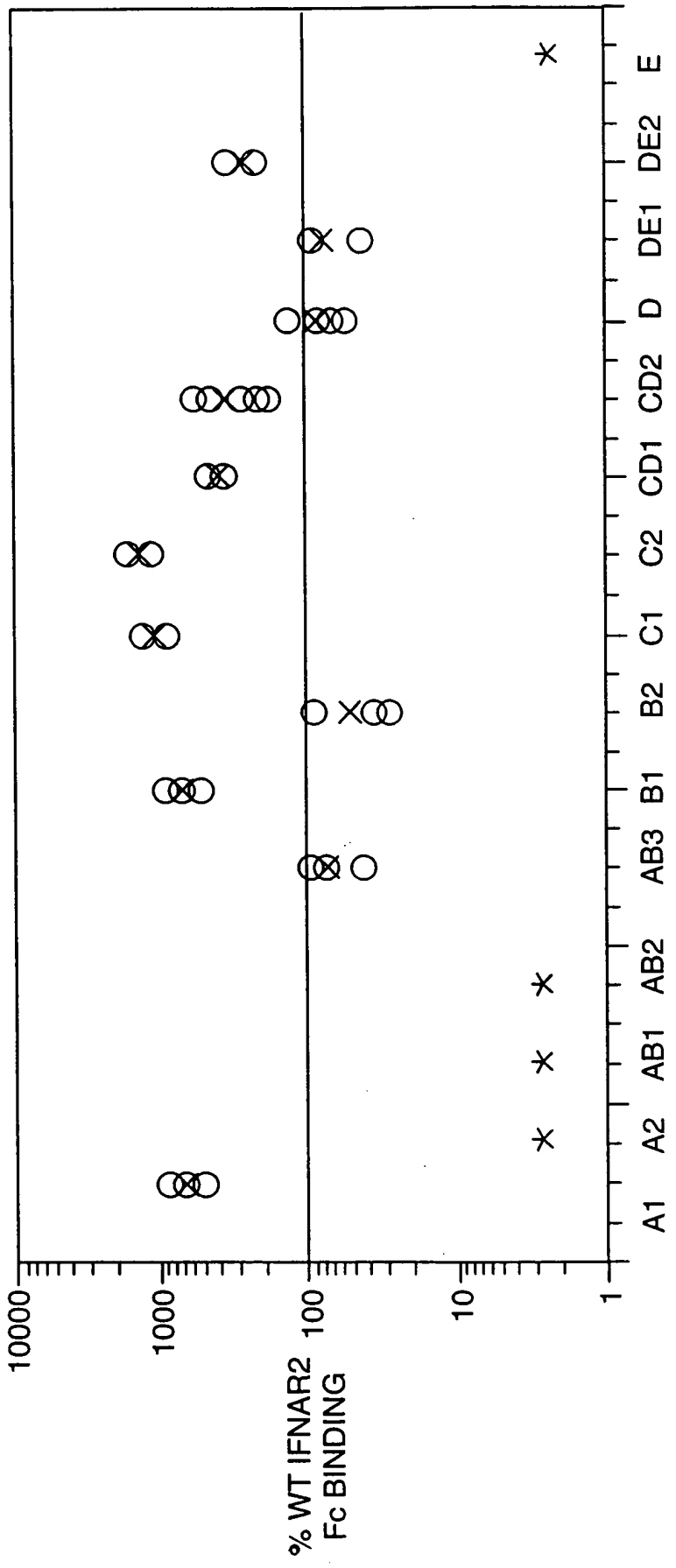
% WT IFNAR2
Fc BINDING
10000
1000
100
10
1
A1
A2
AB1
AB2
AB3
B1
B2
C1
C2
CD1
CD2
D
DE1
DE2
E

FIG. 1

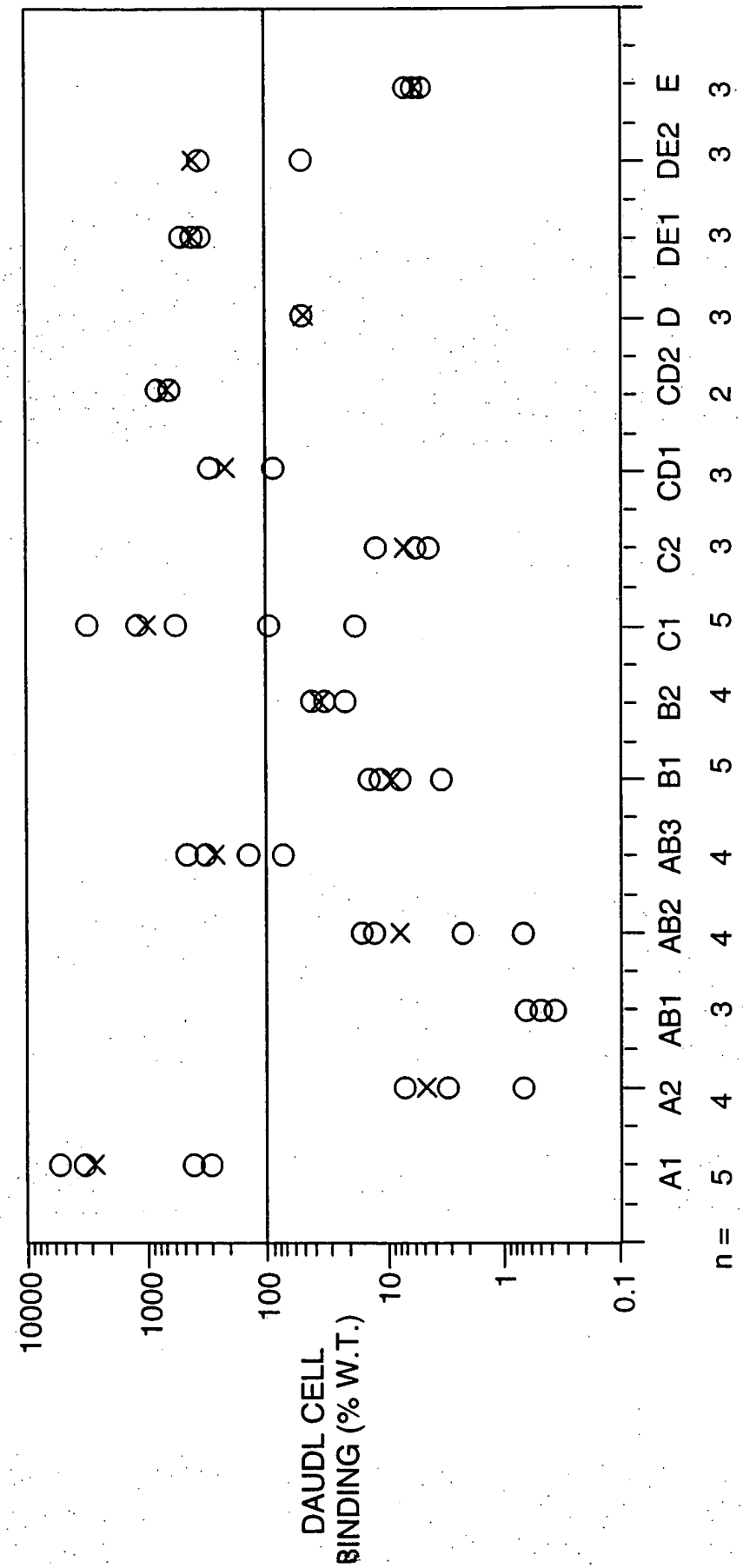
DAUDL CELL
BINDING (% W.T.)
10000
1000
100
10
1
0.1
n = 5 4 3 4 4 5 4 5 3 3 2 3 3 3 3
A1 A2 AB1 AB2 AB3 B1 B2 C1 C2 CD1 CD2 D DE1 DE2 E

FIG. 2

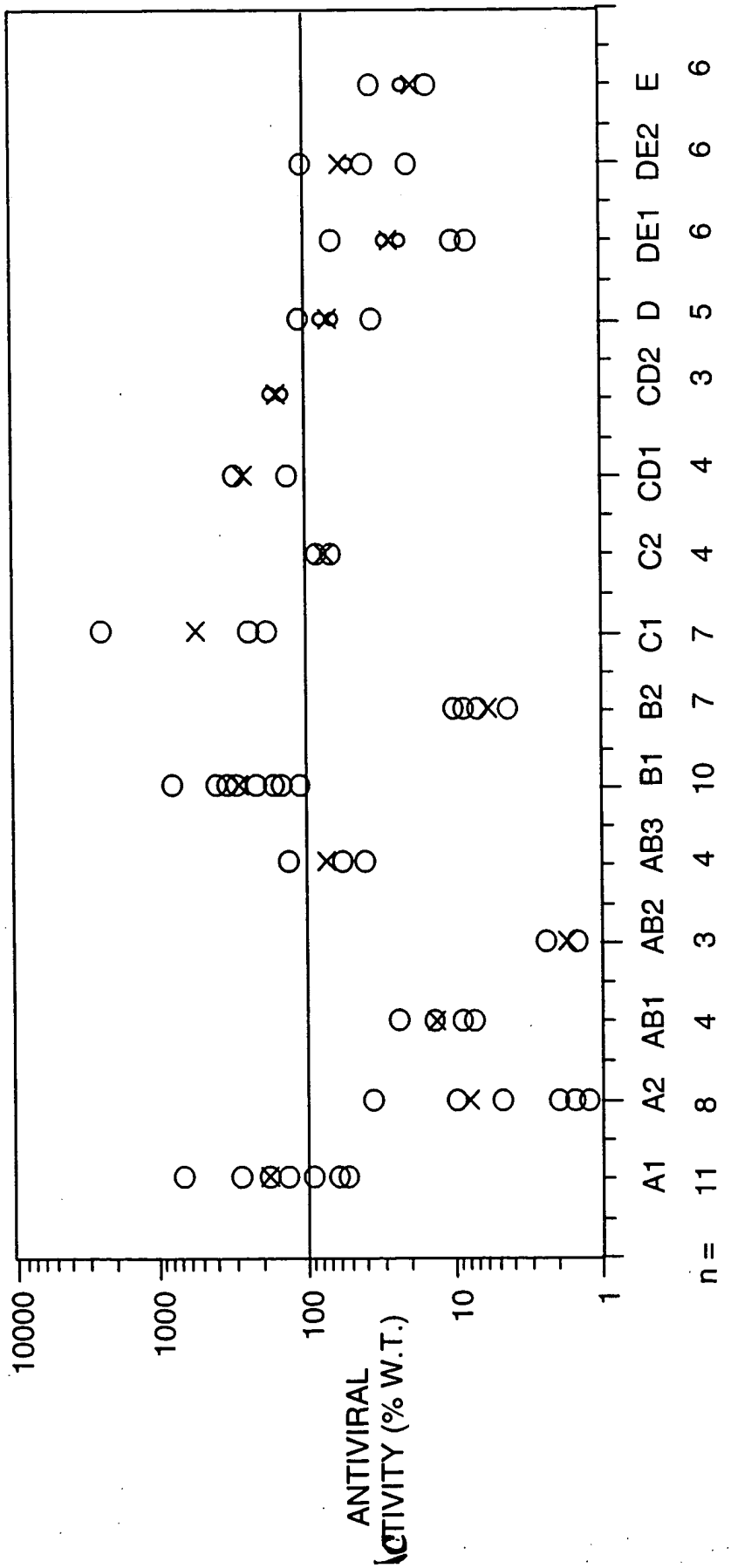
ANTIVIRAL ACTIVITY (% W.T.)
10000
1000
100
10
1
n = 11 8 4 3 4 10 7 4 7 4 3 5 6 6 6
A1 A2 AB1 AB2 AB3 B1 B2 C1 C2 CD1 CD2 D DE1 DE2 E

FIG. 3

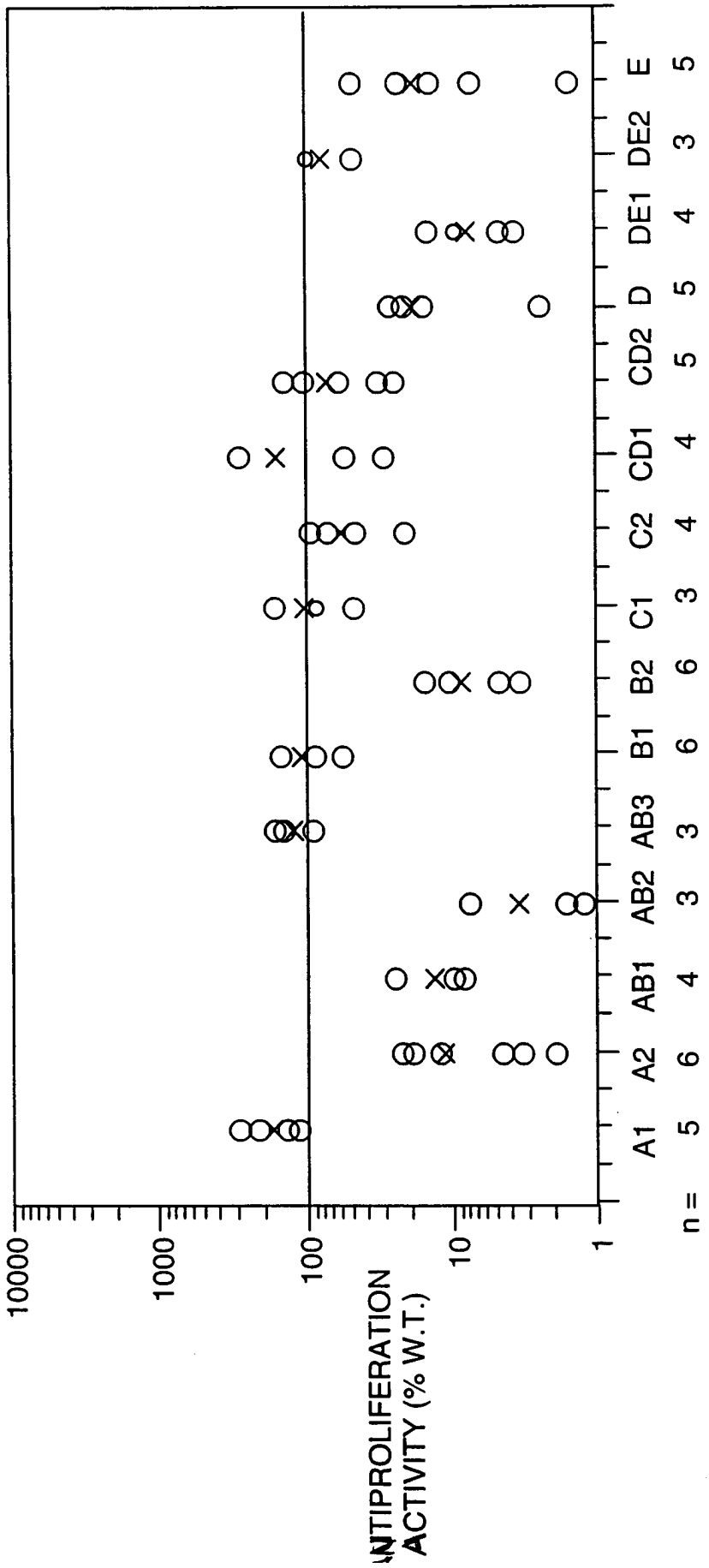
10000
1000
100
10
1
ANTIPROLIFERATION ACTIVITY (% W.T.)
n = 5 6 4 3 3 6 6 3 4 5 5 4 3 5
A1 A2 AB1 AB2 AB3 B1 B2 C1 C2 CD1 CD2 D DE1 DE2 E

FIG. 4

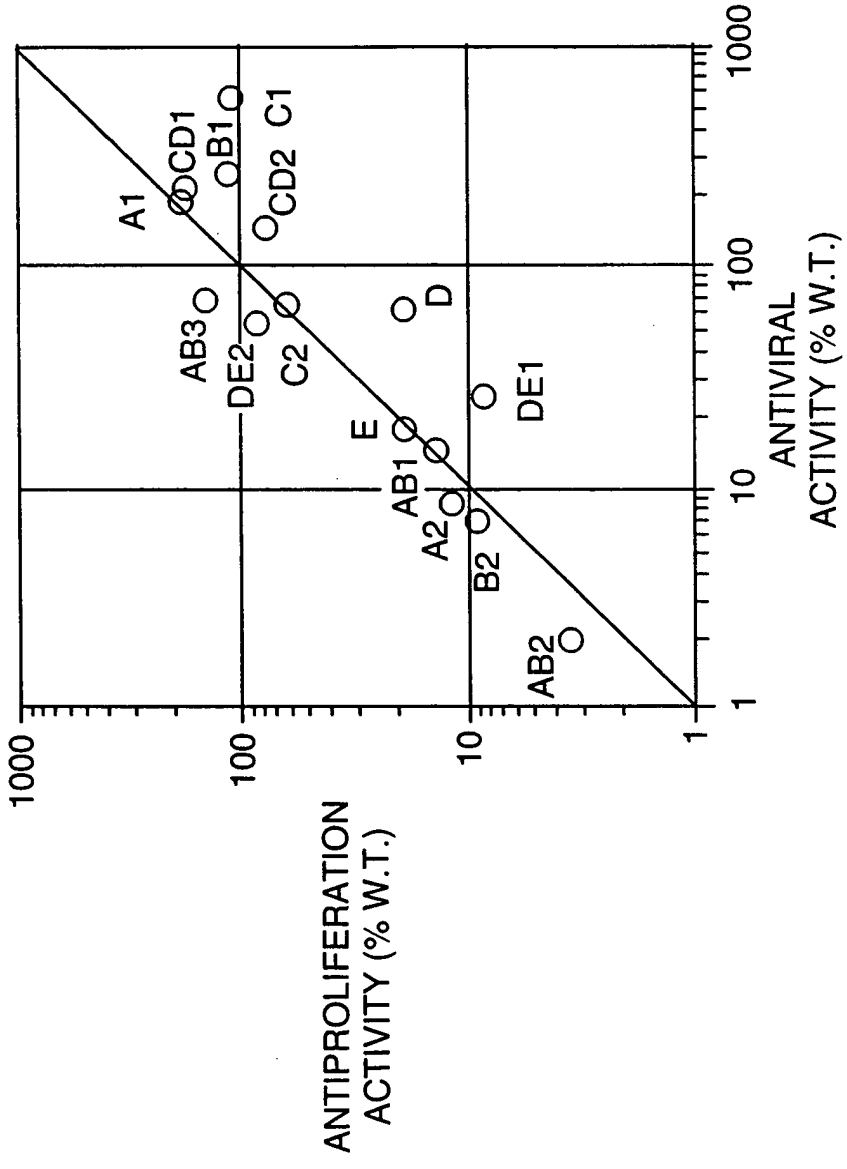
ANTIPROLIFERATION
ACTIVITY (% W.T.)
ANTIVIRAL
ACTIVITY (% W.T.)
1000
100
10
1
1
10
100
1000
A1
CD1
B1
CD2
C1
AB3
DE2
C2
D
E
AB1
A2
B2
DE1
AB2

FIG. 5

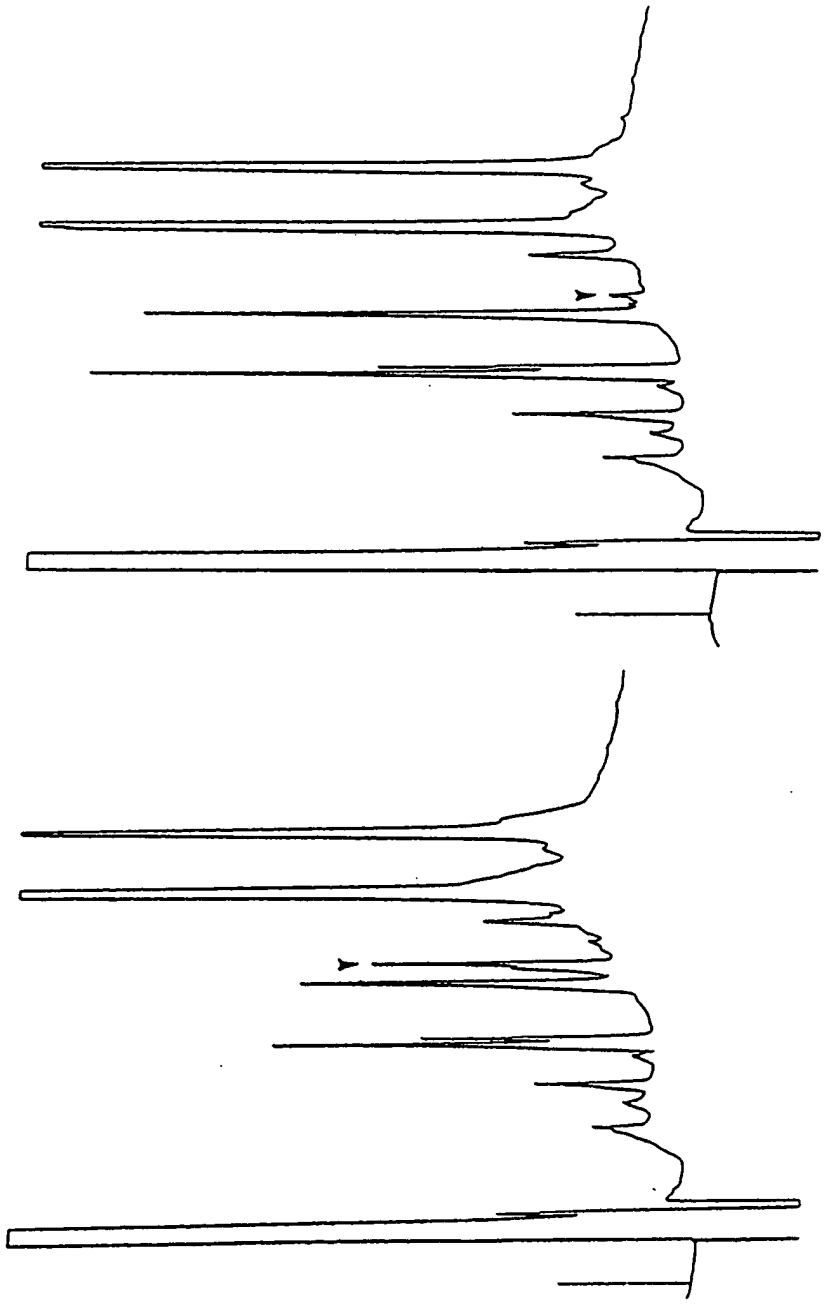

FIG. 6B

FIG. 6A

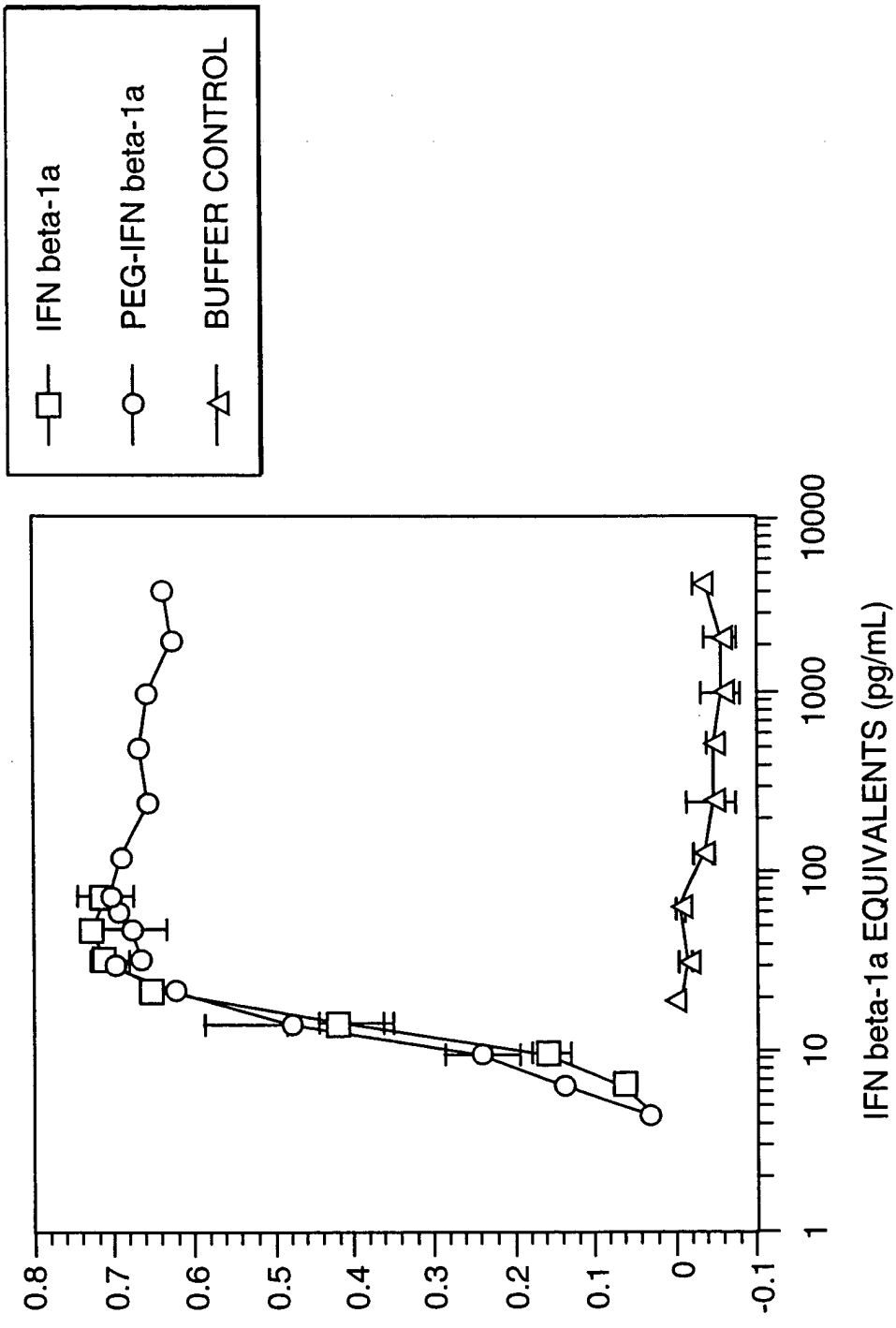
IFN beta-1a
PEG-IFN beta-1a
BUFFER CONTROL
ABSORBANCE (450 nm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
1
10
100
1000
10000
IFN beta-1a EQUIVALENTS (pg/mL)

FIG. 7

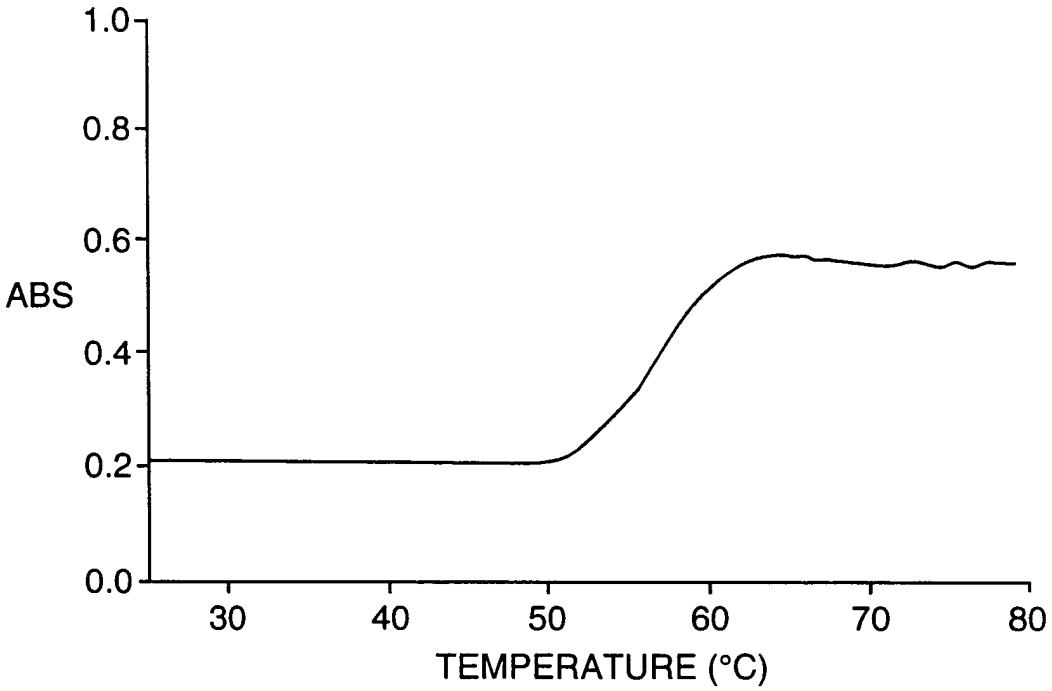
1.0
0.8
0.6
ABS
0.4
0.2
0.0
30
40
50
60
70
80
TEMPERATURE (°C)

FIG. 8a

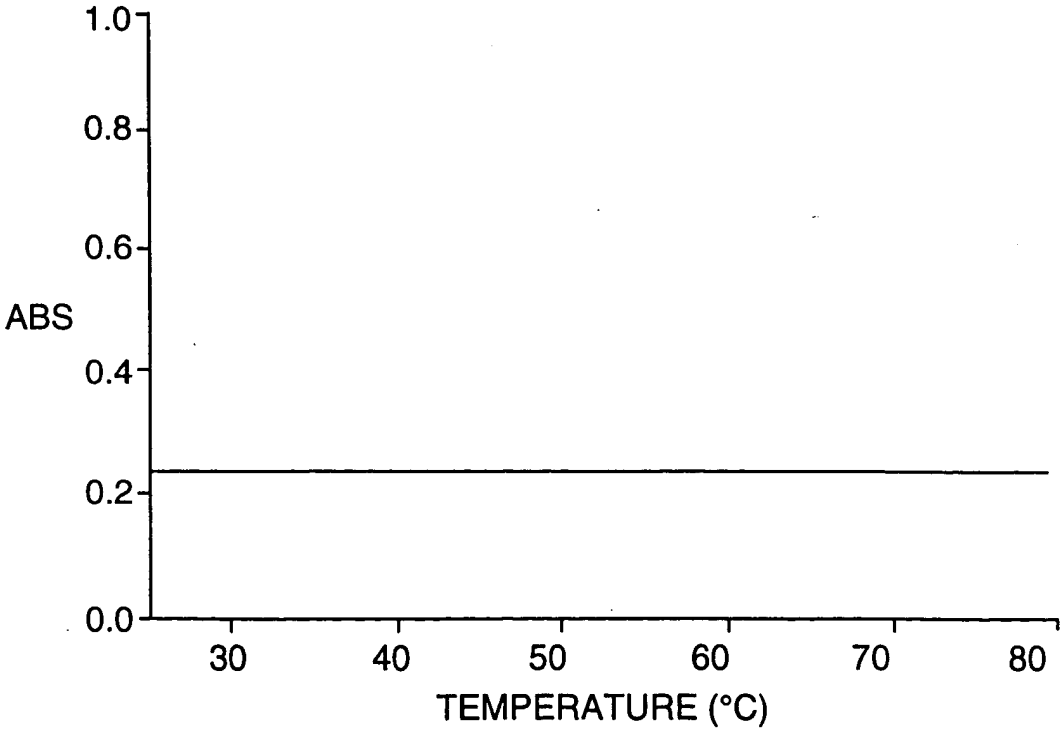
1.0
0.8
0.6
ABS
0.4
0.2
0.0
30
40
50
60
70
80
TEMPERATURE (°C)

FIG. 8b

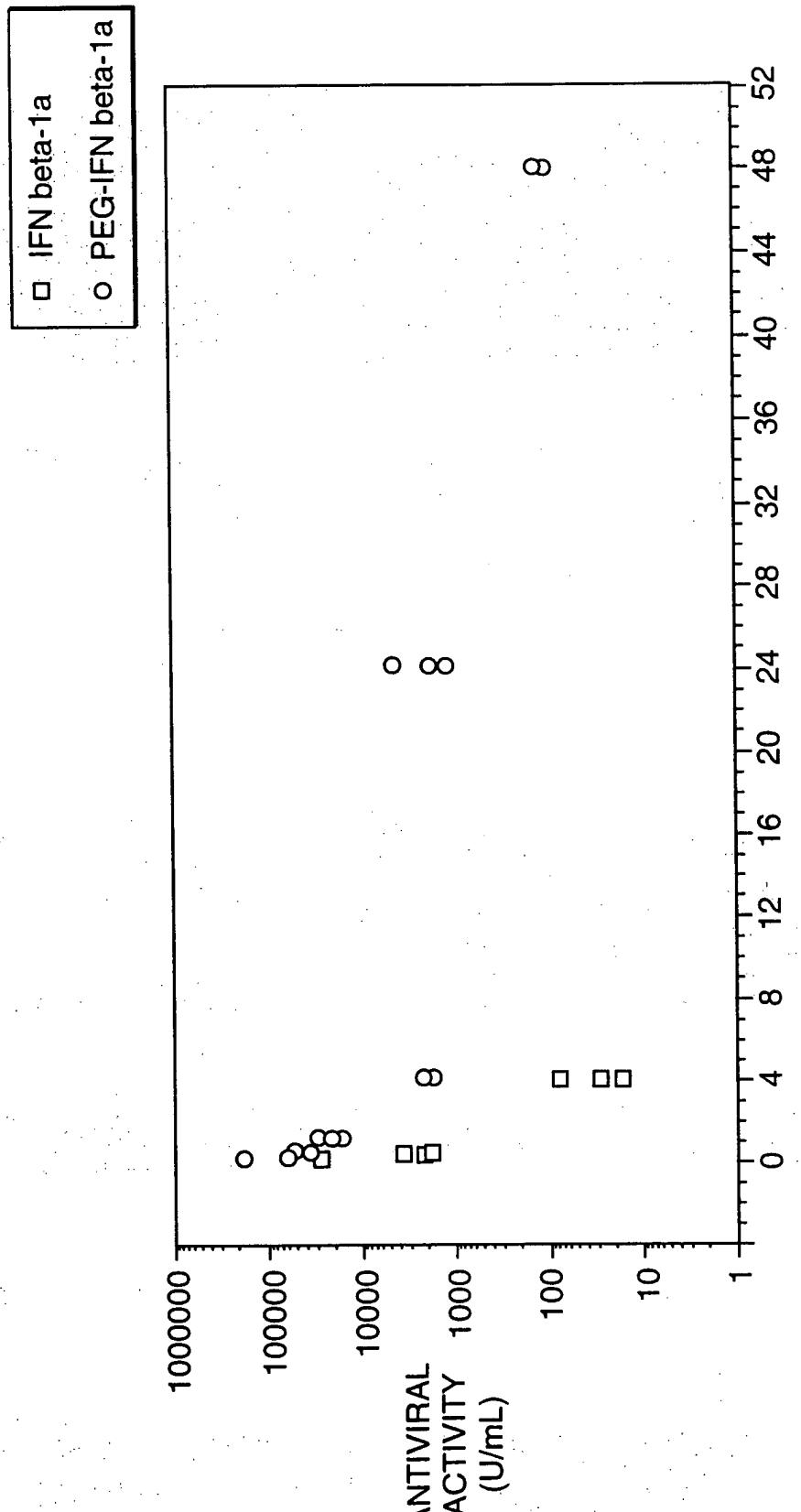
IFN beta-1a
PEG-IFN beta-1a
ANTIVIRAL ACTIVITY (U/mL)
1000000
100000
10000
1000
100
10
1
0
4
8
12
16
20
24
28
32
36
40
44
48
52
TIME AFTER INJECTION (HOURS)

FIG. 9

```
  1 TCCGGGGGCC ATCATCATCA TCATCATAGC TCCGGAGACG ATGATGACAA GATGAGCTAC
    AGGCCCCCGG TAGTAGTAGT AGTAGTATCG AGGCCTCTGC TACTACTGTT CTACTCGATG
  1 ▶SerGlyGlyH isHisHisHi sHisHisSer SerGlyAspA spAspAspLy sMetSerTyr

 61 AACTTGCTTG GATTCCTACA AAGAAGCAGC AATTTTCAGT GTCAGAAGCT CCTGTGGCAA
    TTGAACGAAC CTAAGGATGT TTCTTCGTCG TTAAAAGTCA CAGTCTTCGA GGACACCGTT
 21 ▶AsnLeuLeuG lyPheLeuGl nArgSerSer AsnPheGlnC ysGlnLysLe uLeuTrpGln

121 TTGAATGGGA GGCTTGAATA CTGCCTCAAG GACAGGATGA ACTTTGACAT CCCTGAGGAG
    AACTTACCCT CCGAACTTAT GACGGAGTTC CTGTCCTACT TGAAACTGTA GGGACTCCTC
 41 ▶LeuAsnGlyA rgLeuGluTy rCysLeuLys AspArgMetA snPheAspIl ePr oGluGlu

181 ATTAAGCAGC TGCAGCAGTT CCAGAAGGAG GACGCCGCAT TGACCATCTA TGAGATGCTC
    TAATTCGTCG ACGTCGTCAA GGTCTTCCTC CTGCGGCGTA ACTGGTAGAT ACTCTACGAG
 61 ▶IleLysGlnL euGlnGlnPh eGlnLysGlu AspAlaAlaL euThrIleTy rGluMetLeu

241 CAGAACATCT TTGCTATTTT CAGACAAGAT TCATCTAGCA CTGGCTGGAA TGAGACTATT
    GTCTTGTAGA AACGATAAAA GTCTGTTCTA AGTAGATCGT GACCGACCTT ACTCTGATAA
 81 ▶GlnAsnIleP heAlaIlePh eArgGlnAsp SerSerSerT hrGlyTrpAs nGluThrIle

301 GTTGAGAACC TCCTGGCTAA TGTCTATCAT CAGATAAACC ATCTGAAGAC AGTCCTGGAA
    CAACTCTTGG AGGACCGATT ACAGATAGTA GTCTATTTGG TAGACTTCTG TCAGGACCTT
101 ▶ValGluAsnL euLeuAlaAs nValTyrHis GlnIleAsnH isLeuLysTh rValLeuGlu

361 GAAAAACTGG AGAAAGAAGA TTTCACCAGG GGAAAACTCA TGAGCAGTCT GCACCTGAAA
    CTTTTTGACC TCTTTCTTCT AAAGTGGTCC CCTTTTGAGT ACTCGTCAGA CGTGGACTTT
121 ▶GluLysLeuG luLysGluAs pPheThrArg GlyLysLeuM etSerSerLe uHisLeuLys

421 AGATATTATG GGAGGATTCT GCATTACCTG AAGGCCAAGG AGTACAGTCA CTGTGCCTGG
    TCTATAATAC CCTCCTAAGA CGTAATGGAC TTCCGGTTCC TCATGTCAGT GACACGGACC
141 ▶ArgTyrTyrG lyArgIleLe uHisTyrLeu LysAlaLysG luTyrSerHi sCysAlaTrp

481 ACCATAGTCA GAGTGGAAAT CCTAAGGAAC TTTTACTTCA TTAACAGACT TACAGGTTAC
    TGGTATCAGT CTCACCTTTA GGATTCCTTG AAAATGAAGT AATTGTCTGA ATGTCCAATG
161 ▶ThrIleValA rgValGluIl eLeuArgAsn PheTyrPhel leAsnArgLe uThrGlyTyr

541 CTCCGAAAC
    GAGGCTTTG
181 ▶LeuArgAsn
```

FIG. 10

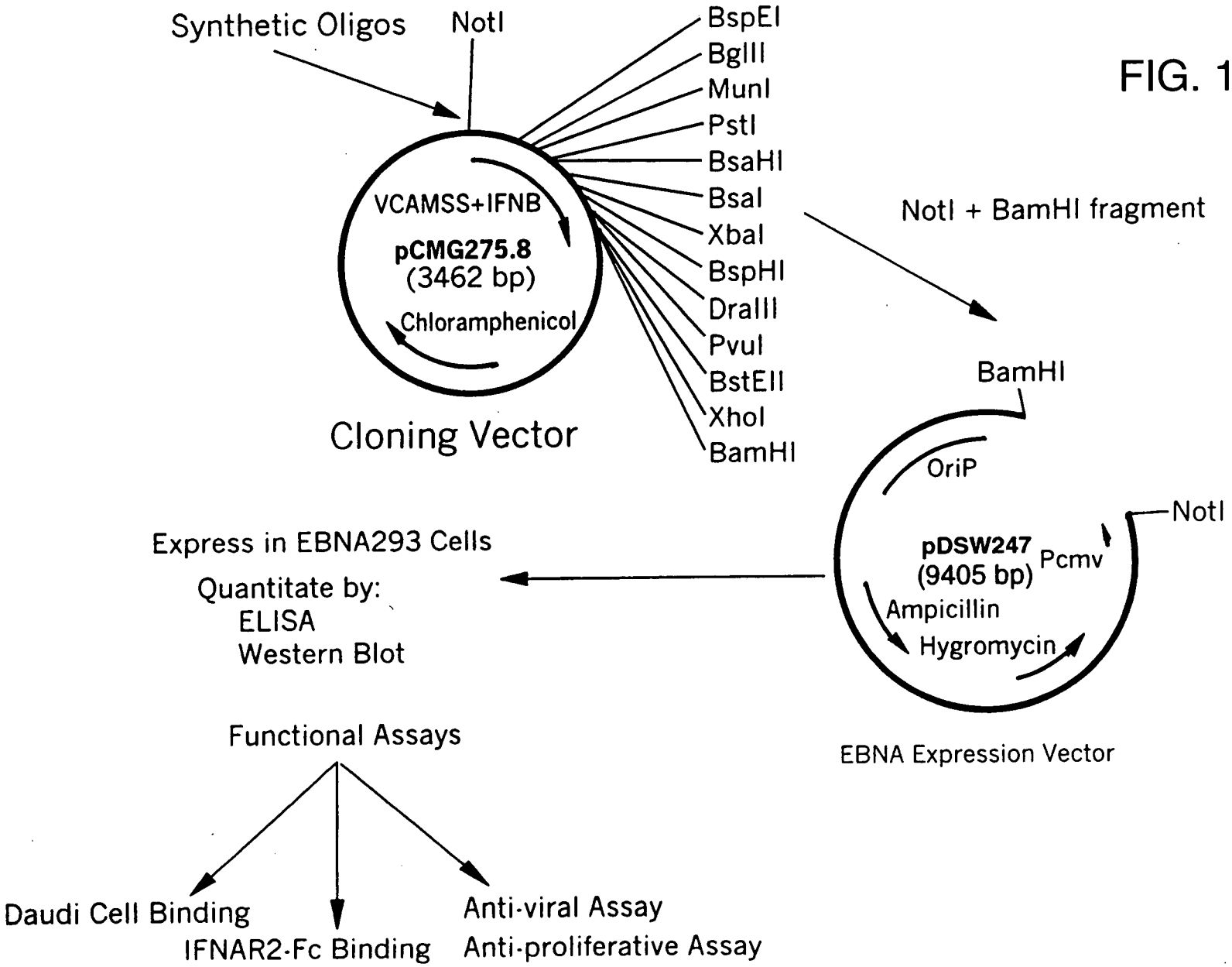
Synthetic Oligos
NotI
BspEI
BglII
MunI
PstI
BsaHI
BsaI
XbaI
BspHI
DraIII
PvuI
BstEII
XhoI
BamHI
VCAMSS+IFNB
pCMG275.8
(3462 bp)
Chloramphenicol
Cloning Vector
NotI + BamHI fragment
BamHI
OriP
NotI
pDSW247
(9405 bp)
Pcmv
Ampicillin
Hygromycin
EBNA Expression Vector
Express in EBNA293 Cells
Quantitate by:
ELISA
Western Blot
Functional Assays
Daudi Cell Binding
IFNAR2-Fc Binding
Anti-viral Assay
Anti-proliferative Assay

FIG. 11